BIOREMEDIATION OF CONTAMINATED WATER SOURCES WITH SULPHATE-REDUCING BACTERIA

D.G. TECLU[1*], M.D. LAING[2] and F.M. WALLIS[1]

[1*]Discipline of Microbiology, University of KwaZulul-Natal,
Private Bag X01, Scottsville, 3209, KwaZulu-Natal, South Africa; E-mail: daniel2003g@yahoo.com
[2]Plant Pathology, University of KwaZulul-Natal,
Private Bag X01, Scottsville, 3209, KwaZulu-Natal, South Africa

ABSTRACT

Bioremediation of arsenic contaminated water by SRB could be a cost-effective process, especially if a suitable carbon source and support matrix were available. To these ends, the chemical composition of molasses was investigated as a candidate for the former purpose while pine bark, sand and polystyrene were assessed as support matrices. Batch culture studies were carried out to assess 1, 2.5 and 5 g l^{-1} molasses as suitable concentrations for SRB growth. The results show that all concentrations supported SRB growth, the response being dependent on the amount present; however, growth on molasses was not as good as that obtained when lactate was used. Biofilm formation on the matrices was evaluated in batch cultures in flasks containing Postgate medium B. The inherent ability of these matrices to support growth of the organisms was evaluated on the basis of pH and redox potential change and the levels of sulphate removal and sulphide production occurring. Environmental scanning electron microscopy (ESEM) was used to characterise the matrix surfaces.

A consortium of SRB growing on polystyrene caused a 49% removal of the original sulphate present whereas on sand a 36% reduction occurred. With pine bark as support matrix no significant sulphate removal occurred. Polystyrene was further examined for its durability as a long-term support material for the growing of SRB in the presence of As(III) and/or As(V) at concentrations of 1, 5 and 20 mg l^{-1}.

An immobilised mixed culture of SRB with molasses as carbon source and polystyrene as support matrix was grown in laboratory-scale bioreactors to investigate the treatment of synthetic groundwater containing either As(III) or As(V) at initial concentration of 20, 10, 5 and 1 mg l^{-1}. Percentage removal of As(III) improved from about 10% to 47% when the concentration was reduced from 20 to 1 mg l^{-1} whereas the corresponding improvement for As(V) was from 39% to 92% during the 14-day experiment.

1. INTRODUCTION

Bioremediation of metal/metalloid-contaminated groundwater makes the use of sulphate-reducing bacteria (SRB) that reduce sulphate to sulphide while oxidising a carbon source (Malik, 2004). The sulphide so generated can remove metals, precipitating them as metal sulphides. The effectiveness of SRB in removing metals from contaminated groundwater depends on the choice of an appropriate organic carbon source for use by the bacteria. The primary consideration when selecting a carbon source is its effect on the extent of microbial activity (biotreatment efficiency) and economic feasibility (Gibert *et al*., 2004).

SRB oxidise organic matter into bicarbonate anaerobically using sulphate as a terminal electron acceptor according to the reaction:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

where CH_2O represents the organic substrate. The hydrogen sulphide generated may form insoluble complexes with many heavy metals such as Ni, Zn, Cu (Poulson *et al*., 1997; Rittle *et al*., 1995).

Molasses is a by-product of sugar processing and can be employed as a relatively cheap carbon source. The composition of molasses can be influenced by a number of factors and contains varying amounts of water, sucrose, glucose, fructose, and non-nitrogenous acids (Paturau, 1989).

Immobilised microbial cells often outperform their planktonic counterparts in the treatment of polluted waters (Singh *et al*., 2006); thus, the immobilisation of SRB on solid support-matrices could improve their performance during the bioremediation of metal contaminated waters. It is important to select a suitable support-matrix for cell immobilisation, especially if sulphate removal is needed (Silva *et al*., 2006). The number and type of microorganisms adhering to the surface may differ from one support to another (Savage and Fletcher, 1985) and as a result may affect the bioremediation efficiency of the system.

Several strategies exist for the treatment of groundwater. Coagulation/filtration, ion exchange, lime softening, adsorption on iron oxides or activated alumina, and reverse osmosis have been used to treat groundwater contaminated with arsenic, particularly As(V) (Zouboulis and Katsoyiannis, 2002). For efficient removal of As(III), an oxidation step may be performed by the addition of chemical reagents such as potassium permanganate, chlorine, ozone, hydrogen peroxide, or manganese oxide prior to applying the above mentioned processes (Kim and Nriagu, 2000).

The objectives of this study were: (1) to determine the chemical composition of molasses and assess its capacity to sustain SRB activity; (2) to investigate the effect of arsenic species [As(III) and As(V)] on the growth of a mixed culture of SRB in a molasses-containing medium; (3) to evaluate the adhesion of a mixed culture containing SRB to pine bark, polystyrene and sand on the basis of sulphate removal efficiency and (4) to investigate the removal of arsenic species from groundwater using bioreactors containing polystyrene immobilised with SRB, molasses as carbon source, and sulphate as electron acceptor. The arsenic was at 20, 10, 5, 1 and 0.1 mg l^{-1} As(III) or As(V) alone or in the ratio As(III):As(V) 0.25:4.

2. MATERIALS AND METHODS

Source of Bacteria

The sulphate-reducing bacteria used in this study were isolated from anaerobic sediments from the Msunduzi River (Pietermaritzburg, South Africa).

Nutrient Media

The nutrient medium (Postgate medium B) (Postgage, 1979) for the growth of sulphate-reducing bacteria contained of (g/l): 0.5 KH_2PO_4, 1 NH_4Cl, 1 $CaSO_4$, 2 $MgSO_4 \cdot 7H_2O$, 3.5 sodium lactate, 1 yeast extract, 0.1 ascorbic acid, 0.1 thioglycollic acid and 0.5 $FeSO_4 \cdot 7H_2O$.

SRB Enrichment

About 50 g of wet sediment was added to a 1 l flask, which was then completely filled with Postgate medium B, sealed with a rubber bung and incubated in the dark at room temperature (25±2°C) for 7 days. After this, 200 ml of the cell suspension was sub-cultured into a new 1 l flask and incubated under identical conditions for a further 7 days. This procedure was repeated every 3 weeks to maintain the SRB culture.

Arsenic Solutions

Arsenic stock solutions [As(III) and As(V)] were prepared by dissolving sodium arsenite ($NaAsO_2$) or sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) in deionised water to a concentration of 1000 mg l^{-1} As. Working solutions were freshly prepared by diluting the stock solutions with appropriate amounts of deionised water as needed.

Synthetic Groundwater

The arsenic-contaminated synthetic groudwater used in this study was prepared by spiking either tap or distilled water with As(III) and/or As(V). The range of concentrations for both forms of arsenic were 20, 10, 5, 1 and 0.1 mg l^{-1}. The major ions present in the test water were (mg l^{-1}), SO_4^{2-} 175, NO_3^- 6.29, Ca 112, Mg 64.4 and Na 102.

Immobilisation Substrates and Source of Molasses

The three materials evaluated as attachment surfaces for the immobilisation of SRB were pine bark, expanded polystyrene (packaging material) and sand. These materials were chosen because of their ready availability and low cost. Total surface area of polystyrene, pine bark and sand (30, 80 and 3400 cm^2 g^{-1} respectively) were estimated using a microscope (Leica) fitted with a digital camera (JVC, model KY-F-1030V) and the software package analySIS. The molasses used as carbon source for the bacteria, was obtained from Voermol Feeds (Pty) Ltd., South Africa.

Bioreactor Configuration and Experimental Set-up

Each bioreactor had a capacity of 12 l. Inner containers with mesh at the bottom and top to disperse the upwards flow of the medium were filled with small pieces of polystyrene (approximately 10-15 mm × 12-16 mm × 9-12 mm) as support matrices. The bioreactors were inoculated with a SRB culture containing ~3×10^5 cells ml^{-1} (20% vol vol^{-1}). The void volume in the inner containers when filled with polystyrene was approximately 4.2 l. Figure 1 shows a schematic diagram of a bioreactor and its dimensions.

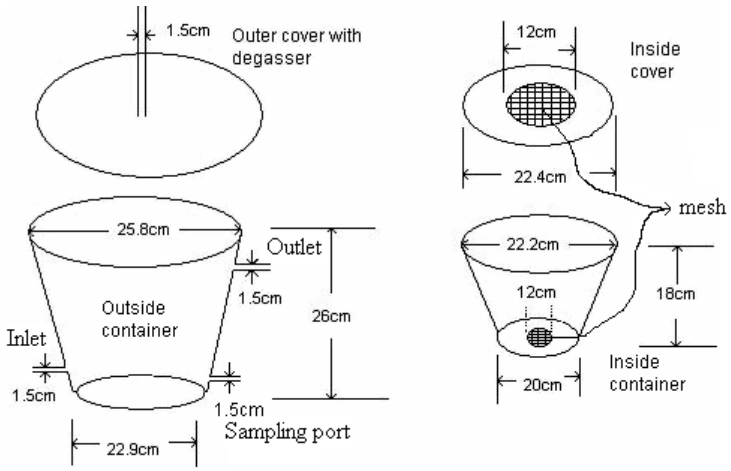

Figure 1. Schematic diagram of the plastic bioreactor. The inner container, which fits inside the outer one, is packed with the different biomass support matrices.

Molasses served as the carbon source, polystyrene as support matrix and sulphate as the electron acceptor.

Sulphate and Sulphide Analysis

A modified turbidimetric method was used to measure the residual sulphate concentration (Kolmert et al., 2000). Sulphide was analysed spectrophotometrically by measuring methylene blue formed from the reaction of the ion with N-N-dimethyl-1,4-phenylenediammonium dichloride (Cline, 1969).

Arsenic Analysis and Speciation

Arsenic species were analysed using hydride generation coupled to an ICP detection system according to a modified method developed by Müller (1999).

Experimental Cultures

Experimental cultures were grown with different concentrations of molasses (1, 2.5 and 5 g l^{-1}) as carbon source. The growth studies were performed in duplicate using a 20% (v v^{-1}) inoculum of log phase cells that had been sub-cultured 3 times. All cultures were incubated in the dark at room temperature (25±2°C) for 1-2 weeks. Growth of the SRB was monitored microscopically by direct cell counts and verified by measuring sulphate removal levels. As a control, the same medium was used but with lactate as carbon source as most sulphate reducers can metabolise this compound.

Arsenic Tolerance Study

The influence of different concentrations of As(III) and As(V) on growth of the SRB consortium was studied. Appropriate volumes of either arsenate or arsenite were added to the culture medium from stock solutions to give final concentrations of 1, 5 and 20 mg l^{-1}. Controls contained the same growth media but without arsenic. The effect of the arsenic species was evaluated using a sulphate activity assay (involving sulphate removal kinetics and measures maximum sulphate removal level).

Immobilisation of SRB

Flasks containing Postgate medium B and either pine bark, polystyrene or sand were inoculated with a 20% inoculum of a pre-grown mixed culture of SRB to study cell immobilisation. Polystyrene immobilised cultures performed best and hence this support matrix was further investigated.

Statistical Analysis

Data was analysed using analysis of variance (ANOVA) by Genstat (10^{th} edition) program.

3. RESULTS AND DISCUSSION

At a concentration of 1 g l^{-1} molasses, the pH increased slightly from 6.4 to 6.9 over a 14-day period. Over the same period, but with molasses at 2.5 g l^{-1} and 5 g l^{-1} the pH increased to 7.0 and 7.1 respectively.

There was no significance difference in all cell growth or medium pH at the various molasses concentrations investigated.

In parallel with the pH changes, the redox potential of the medium declined from 254 mV to -179 mV over 14 days for 1 g l^{-1} molasses; from 248 mV to -195 mV for 2.5 g l^{-1} molasses; and from 235 mV to -210 mV for 5 g l^{-1} molasses. However, the corresponding change when lactate was used as carbon source was from 245 mV to -269 mV. There was a slight difference in redox potential for the different molasses concentrations at the start of

the experiments.

The percentage sulphate reduced during the 14-day batch culture experiments on SRB growth with different concentrations of molasses as carbon source is shown in Figure 2. The graph show that the rate of sulphate removal was higher in the control (lactate) flasks than in the flasks containing the three molasses concentrations. The percentage removal was fairly similar in all the molasses-containing flasks.

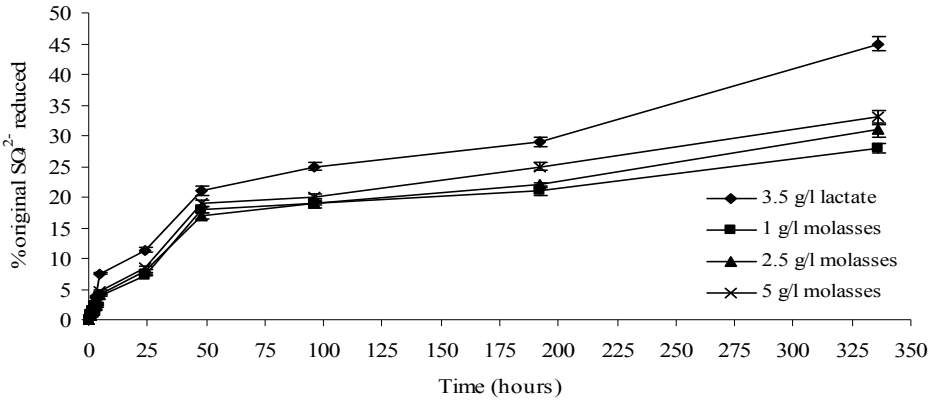

Figure 2. Percentage SO42- removal as a function of time during the growth of SRB on 1, 2.5 and 5 g l-1 molasses and 3.5 g l-1 lactate. Error bars represent standard deviation between 3 measurements. Statistically non-significant at P=0.05.

The increase in pH observed when either molasses or lactate were used as carbon source for growth of SRB reflects the oxidation of the organic carbon (electron donor) source into bicarbonate thereby increasing the alkalinity. The increase in pH and accompanying decrease of redox potential during bacterial growth possibly indicate the establishment of anaerobic reducing conditions which are conducive to the growth of SRB. Sulphate removal by SRB occurs when the redox potential is below -100 mV (Postgate, 1979).

Effect of Arsenic Species on the Growth of SRB

Arsenite and arsenate had a negative effect on the SRB, the growth rate being slower as the concentrations of both arsenic species increased from 1 mg l^{-1} to 20 mg l^{-1}. The duration of the lag phase also increased with increasing concentrations of each arsenic species, indicating that at high concentrations of arsenite and arsenate the growth of SRB was inhibited.

Figures 3 and 4 show the percentage sulphate removal occurring in the presence of different concentrations of As(III) and As(V), respectively. For the control and for both arsenic species at 1 mg l^{-1}, the removal of sulphate reached 5% on day 3 and thereafter increased at a roughly uniform rate. At 5 mg l^{-1} of either arsenic species the 5% removal level was reached only on day 5. This indicated that at high concentrations of either arsenic species the ability of SRB to reduce sulphate to sulphide was decreased from 49% to 7% As(III) and 12% As(V).

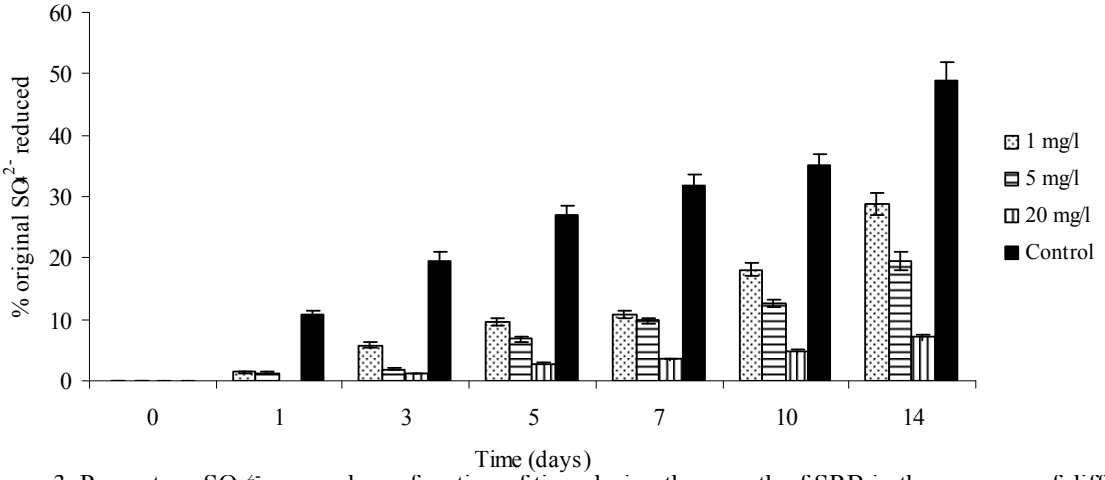

Figure 3. Percentage SO_4^{2-} removal as a function of time during the growth of SRB in the presence of different As(III) concentrations. Error bars represent standard deviation between 3 measurements. Statistically significant at P=0.05.

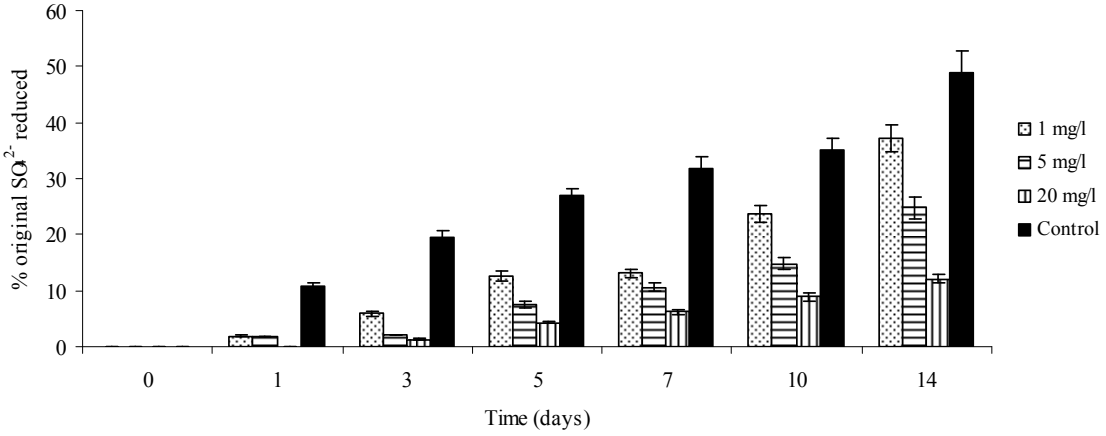

Figure 4. Percentage SO_4^{2-} removal as a function of time during the growth of SRB in the presence of different As(V) concentrations. Error bars represent standard deviation between 3 measurements. Statistically significant at P=0.05.

Regardless of the initial concentration of arsenic, sulphate removal was always greater in the presence of As(V) than in the presence of As(III).

Immobilisation of Srb

PH and Redox Potential

Initially, the pH remained constant in all SRB-inoculated flasks except those containing pine bark as the support material. A very slow increase in pH occurred in the flasks containing sand and polystyrene and in the matrix-free control flasks after day one, whereas with pine bark the pH decreased fairly dramatically throughout the experiment. After 10 days, the pH in the polystyrene, sand and matrix-free system was approximately 7.2, 7.2 and 7.1 respectively whereas with pine bark it was 4.6. Similarly, the redox potential started to decrease from day one in the polystyrene - and sand-immobilised and in the free-living SRB cultures but not in the pine bark culture. At day 10, the redox potential in the polystyrene, sand and matrix-free cultures was -227, -205 and -195 mV respectively. In contrast the redox potential in the pine bark immobilised culture at day 10 was only -19 mV.

Sulphate Removal

Sulphate reduction by the cells growing on polystyrene was superior (49%) to that by cells immobilised on the other materials tested (viz. sand 36%, pine bark ~7%). The low level for pine bark was possibly due to the leaching of toxic chemicals, such as phenolics, that are known to inhibit the growth of SRB (Barkay and Schaefer, 2001). In the free-living culture, sulphate removal amounted to about 42% (Figure 5).

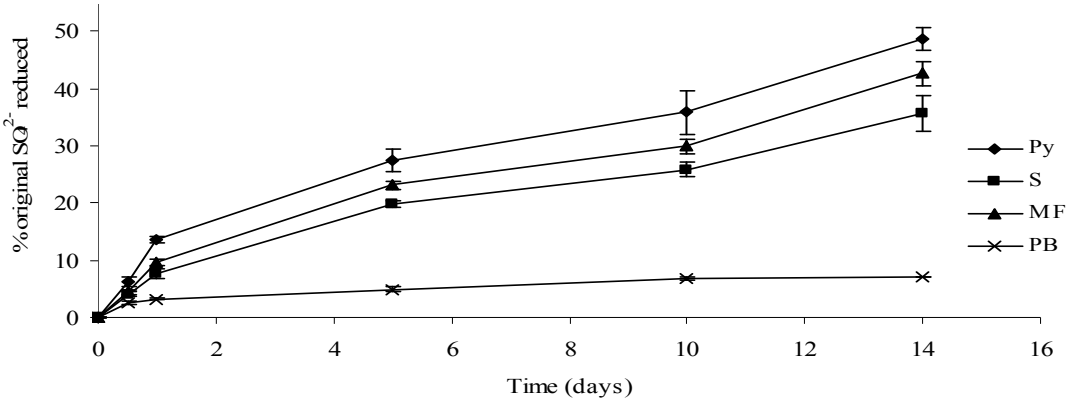

Figure 5. Percentage SO_4^{2-} removal as a function of time (days) during growth of SRB on polystyrene (Py), sand (S), pine bark (PB) and in a matrix-free (MF) culture. Error bars represent standard deviation between 3 measurements. Statistically non-significant at P=0.05.

ESEM photomicrographs showed that the SRB had successfully colonised on the polystyrene support matrix (Figure 6) while growing on molasses.

Figure 6. ESEM micrographs of SRB colonising the polystyrene surface.

The removal efficiencies for arsenic species during the growth of polystyrene-immobilised and free-living SRB were studied. Figures 7, 8 and Table 1 show the changes in concentration of As(III), As(V) and in mixtures of As(III) and As(V), respectively. Both As(III) and As(V) were removed by the mixed culture of SRB either in the presence or absence of the support matrix. Irrespective of the initial concentration, the removal efficiency of As(III) was always inferior to that of As(V). Percentage removal of As(III) improved from about 10% to 47% when the concentration was reduced from 20 to 1 mg l^{-1} (Figure 7) whereas the corresponding improvement for As(V) was from 39% to 92% removal (Table 1) during the 14-day experiment in the immobilised system. In the free-living cell systems, the percentage removals at the end of the 14 day experiment was 43, 33, 12 and 12% for initial As(III) concentrations of 1, 5, 10 and 20 mg l^{-1} respectively whilst for As(V) the corresponding removal values were 88, 76, 69 and 34%. A Bioremoval mechanism of arsenic species is given by Teclu *et al*. (2008)

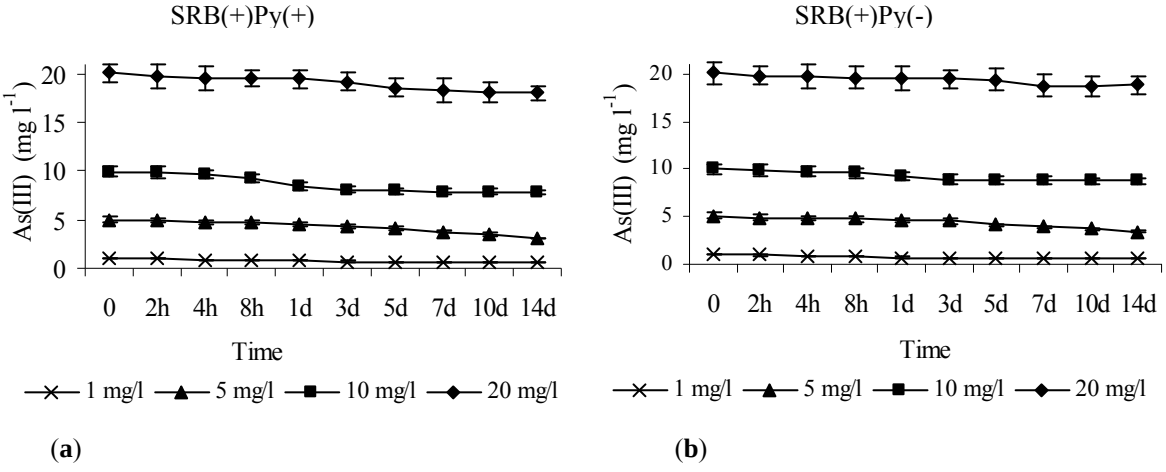

Figure 7. Changes in As(III) concentration as a function of time in the presence of: (a) SRB with polystyrene as support matrix [SRB(+) Py(+)] and (b) SRB without polystyrene [SRB(+) Py(-)].

Table 1. Changes in As(V) concentration as a function of time in the presence of: (a) SRB with polystyrene as support matrix [SRB(+) Py(+)] and (b) SRB without polystyrene [SRB(+) Py(-)].

As(V)/mg/l SRB(+)Py(+)	Time									
	0	2h	4h	8h	1d	3d	5d	7d	10d	14d
20	20.06	19.17	18.47	17.97	17.54	16.52	16.01	15.49	14.73	12.18
10	10.07	9.28	8.59	7.58	6.57	5.37	4.48	3.87	3.07	2.59
5	5.02	4.26	4.07	3.78	3.21	2.49	2.05	1.91	1.16	0.87
1	1.07	0.86	0.64	0.55	0.39	0.25	0.27	0.21	0.10	0.09
As(V)/mg/l SRB(+)Py(-)	Time									
	0	2h	4h	8h	1d	3d	5d	7d	10d	14d
20	20.02	19.21	18.67	18.09	17.76	16.83	16.49	16.07	15.12	13.19
10	10.03	9.33	8.76	8.06	7.06	6.57	4.97	4.28	3.68	3.06
5	4.98	4.33	4.23	3.98	3.65	2.73	2.57	2.16	1.49	1.19
1	1.05	0.91	0.73	0.69	0.51	0.37	0.35	0.29	0.18	0.13

When the total arsenic concentration (i.e., As(III) + As(V) in different proportions) was 100 μg l^{-1}, the removal efficiencies were improved for both As(III) and As(V) and the percentage removal was 52%, 73% and 96% at the end of the 14 day experiment when As(III) comprised 100%, 60% and 0% of the total arsenic respectively (Figure 8). When the residence time was increased to 21 days, the solutions containing 40% As(III) or less (i.e., 40 μg l^{-1} As(III) or less in a total arsenic concentration 100 μg l^{-1}) were efficiently bioremediated to below the WHO acceptance limit of 10 μg l^{-1} (Figure 8).

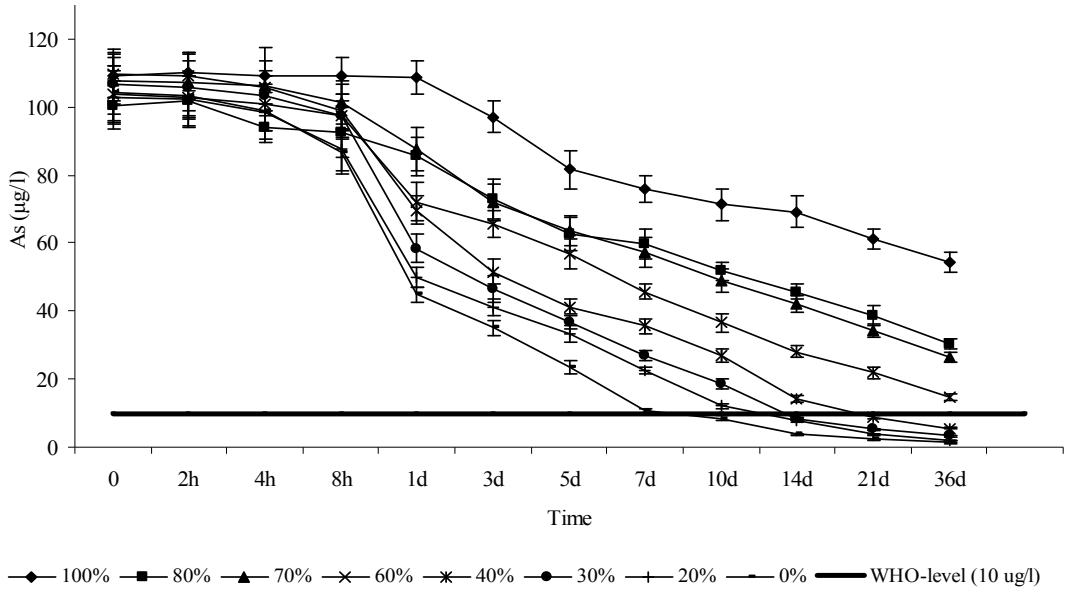

Figure 8. Changes in total arsenic concentration in solutions with different ratios of As(III):As(V) as a function of time in the presence of polystyrene-immobilised SRB.

It is evident that As(V) was removed more efficiently than was As(III) (see Figure 8). Hence, MnO_2 was used to pre-oxidise As(III) to As(V). MnO_2 was the oxidant of choice since Ghurye and Clifford, (2001) recommended it for the treatment of drinking water prior to the removal of arsenic. Our results showed that MnO_2 did oxidise As(III) and that the oxidation rate increased with increasing concentration of the oxidant. Radu *et al* (2008) hypothesised that MnO_2 consists of oxidative sites and non-oxidative sorption sites. The oxidative sites are renewable, and they rapidly oxidise As(III) and release As(V) to the solution through the mechanism postulated by Scott and Morgan (1995)

4. SUMMARY

Molasses as carbon source supported growth of SRB at concentrations of 1, 2.5 and 5 g l^{-1}. This growth was accompanied by an increase in pH and decrease in redox potential.

At 20 mg l^{-1}, both arsenic species, but particularly As(III), reduced the growth of SRB. Likewise, sulphate removal was reduced to less than 8% at this concentration of either As(III) or As(V). At lower concentrations of either arsenite or arsenate, the growth of SRB was considerably better than at higher levels but a prolonged lag phase was evident.

Our results show that the sulphate removal kinetics of immobilised SRB cultures were affected by the support matrix used, with greater sulphate removal occurring in cell populations attached to polystyrene than to sand or pine bark.

Arsenite removal in bioreactors containing a population of SRB immobilised on polystyrene was only about 10% when the initial concentration was 20 mg l^{-1}; the result for the same initial concentration of As(V) was 39%. Planktonic SRB cultures removed less As(III) and As(V) than their immobilised counterparts. The presence of SRB improved the arsenic removal capacity of the system. The efficiency of As(III) removal by the bacteria was enhanced by first oxidising it to the less toxic As(V) using MnO_2.

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